PERFORMANCE OF PHOTO-CURABLE METAL-CHELATING COATING IN COMPLEX FOOD MATRICES

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ABSTRACT

Many packaged foods and beverages undergo transition metal-catalyzed oxidative degradation which causes food spoilage and ultimately food waste and loss. Recently, a nonmigratory photo-curable metal-chelating active packaging material was developed that provides a novel approach to inhibit oxidation of foods. The objective of this study was to investigate the influence of competing ions (Ca^{2+}, Mg^{2+}, Na^{+}) and increasing viscosity on the iron chelation capacity and antioxidant efficacy of metal chelating film in a model system designed to simulate complex conditions typical of food and beverage systems. Iron chelation was quantified by material-bound iron content using inductively coupled plasma-mass spectroscopy (ICP-MS). In the presence of calcium and magnesium, there was a significant decrease in iron chelating capacity; however, materials retained 61% chelating capacity in the presence of 0.8 M sodium. Materials retained iron chelating capacity even with the increase of viscosity to 2700 cP, similar to the viscosity of salad cream. Additionally, the relationship between the competing ions, viscosity and antioxidant efficacy of metal chelating film was studied. Metal chelating film significantly slowed down transition metal-catalyzed ascorbic acid degradation by rising the retained ascorbic acid from 20% to 70% even in the presence of calcium, magnesium, and sodium, and to 61% at increasing viscosity after 7 days storage at 37 °C and pH 4.0. These results suggest that metal chelating active packaging can retain performance as an antioxidant system even in the presence of competing ions or increasing viscosity, and may present a new technological approach to addressing consumer demands for reduced additive use.

BIOGRAPHICAL SKETCH

Pei Zhu is a professional master's student in Food Science at Cornell University. Pei was born in 1995 in China and came to the U.S. in 2015 for a 2+2 double degree program. She received her Bachelor of Engineering in Food Engineering from South China University of Technology and Bachelor of Science in Food Science from Rutgers University in 2017, with the honor of Magna Cum Laude. After that, Pei worked as a quality control intern in Pepsi-Cola Beverage Inc. in China. She was also the graduate teaching assistant in food chemistry laboratory for the fall semester 2017 at Cornell. Her graduate research focus is on nonmigratory metal-chelating active packaging with her advisor Dr. Julie Goddard.

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LIST OF ABBREVIATIONS

ATR−FTIR	Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy
BPM	4-Benzoyphenyl Methacrylate
DI	Deionized
EDTA	Ethylenediaminetetraacetic Acid
GMA-IDA	2-Propenoic Acid, 2-Methyl-,3-[Bis-(Carboxymethyl) Amino]-2-Hydroxypropyl Ester
GMA-IDA-co-BA-co-BPM	Poly (2-Propenoic Acid,2-Methyl-,3-[Bis-(Carboxymethyl) Amino]-2-Hydroxypropyl Ester-Co-N-Butyl Acrylate-Co-4-Benzoylphenyl Methacrylate
ICP-MS	Inductively Coupled Plasma-Mass Spectroscopy
IDA	Iminodiacetic Acid
MC	Methylcellulose
SEM	Scanning Electron Microscopy

INTRODUCTION

Oxidative degradation catalyzed by transition metals (e.g. iron, copper) is a significant issue in the food industry. It can cause a loss in nutritional quality of foods and quality deterioration such as discoloration and the generation of rancid off-flavors in many packaged foods during transportation and storage (Decker, Elias, & McClements, 2010). Indeed, even trace amounts of iron can initiate oxidation of labile molecules such as unsaturated fatty acids, antioxidants, carotenoids and even essential amino acids (Goddard, McClements, & Decker, 2012). To order to prevent spoilage and extend shelf life, synthetic metal chelators such as ethylenediaminetetraacetic acid (EDTA) are commonly added to packaged foods to inhibit such oxidative reactions. However, as consumers are increasingly demanding products with fewer additives and longer shelf-life, alternative preservation technologies are needed (Bearth, Cousin, & Siegrist, 2014).

One strategy for maintaining product quality and shelf life is the design a suitable packaging technology. Packaging plays an essential role in preserving food quality and reducing food waste. It serves as a container for the food and acts as a barrier to protect the food from environmental influences such as oxygen, light, dust, light, and both chemical and microbiological contamination (Yildirim et al., 2018). Packaging design has a close relationship with food waste and the outcome of proper packaging attributes helps avoid food losses from field to fork (Wikström, Williams, Verghese, & Clune, 2014). To meet consumer and retail demands for clean label products, non-migratory metal-chelating active packaging films have been developed, with the aim of removing the synthetic additive EDTA without compromising the product quality and shelf life. In metal-chelating active packaging, chelating ligands are immobilized onto the surface of traditional packaging materials (e.g. polypropylene) via a covalent linkage such that it retains activity but is

unlikely to migrate to the product (Arrua, Strumia, & Nazareno, 2010; Tian, Decker, & Goddard, 2013). Such active packaging technology may thus enhance product shelf-life or maintain or improve the condition of packaged foods without the addition of synthetic additives.

Recently, our research group has developed a new method to produce nonmigratory metal chelating active packaging material in which a latex poly (n-butyl acrylate) based copolymer is synthesized via emulsion copolymerization with iminodiacetic acid (IDA) as metal-chelating ligand and benzophenone as photo-cross-linker (Lin & Goddard, 2018b). Metal chelating active packaging materials can be prepared by coating the copolymer onto polypropylene surface and undergoing UV-curing. Lin and Goddard (2018b) demonstrated the efficacy of this coating in chelating iron and controlling ascorbic acid degradation in acidic aqueous environments (pH 3.0 and 5.0). However, its performance in complex conditions typical of food systems (e.g. presence of competing ions and high viscosity) has not yet been shown.

In another non-migratory metal chelating active packaging technology, in which poly-hydroxamic acids served as the chelating ligand, it was found that iron chelating capacity was decreased with increasing viscosity (Roman, Decker, & Goddard, 2015) but retained chelating capacity in the presence of calcium, magnesium, and sodium competing ions (Ogiwara, Roman, Decker, & Goddard, 2016). In this context, it is hypothesized that the new coating would retain its metal-chelating efficacy despite the influence of competing ions and increasing viscosities.

The goal of the present work was to characterize the influence of competing ions (calcium, magnesium, and sodium) and varying viscosities typical of food and consumer products on the iron-chelating capacity of a non-migratory metal chelating active packaging material prepared by photocuring of an iminodiacetic acid chelating copolymer. Additionally, the antioxidant

performance of the metal-chelating film in controlling ascorbic acid degradation in the presence of competing ions and thickening agent methylcellulose was also determined.

MATERIALS & METHODS

MATERIALS

Iminodiacetic acid (98+%) was purchased from Acros Organics (Morris Plains, NJ). Polypropylene pellets (isotactic) were purchased from Scientific Polymer Products (Ontario, NY). Isopropanol, acetone, hydrochloric acid (trace metal grade), sodium acetate trihydrate, ferric chloride hexahydrate, and ethylenediaminetetraacetic acid (EDTA) (disodium salt dihydrate) were purchased from Fisher Scientific (Fair Lawn, NJ). 2,6-dichloroindophenol sodium salt hydrate, n-butyl acrylate (99+%), potassium persulfate (99+%), L-ascorbic acid, imidazole, sodium chloride, oxalic acid, methylcellulose, magnesium chloride hexahydrate, and calcium chloride dihydrate were purchased from Sigma-Aldrich (St. Louis, MO). Absolute ethanol was purchased from Pharmco-Aaper (Brookfield, CT). Nitric acid (trace metal grade) was purchased from VWR Chemicals (Radnor, PA). All chemicals and reagents were used without further purification. 2-propenoic acid, 2-methyl-,3-[bis-(carboxymethyl) amino]-2-hydroxypropyl ester (GMA-IDA) and 4-benzoyphenyl methacrylate (BPM) were synthesized according to the reported methods (Chuh-Yean & Chuh-Yung, 2002; Lin & Goddard, 2018b; Schlemmer, Betz, Berchtold, Rühe, & Santer, 2009).

METHODS

Metal-chelating film synthesis. To prepare polypropylene films, polypropylene pellets were washed by sonicating in isopropanol, acetone, and deionized (DI) water sequentially and drying in a desiccator (20°C, 15% relative humidity). Dried polypropylene pellets were hot pressed (Carver Laboratory Equipment, Model #3925, Carver Inc., IN) into polypropylene films with 9000 lbs. press force at 200°C. After that, polypropylene films were cut into 5 x 5 cm^2 pieces, cleaned by sonicating in isopropanol, acetone, and DI water, and then dried in a desiccator. The photo-curable

metal-chelating copolymer was synthesized using an emulsion copolymerization of GMA-IDA, n-butyl acrylate and BPM monomers according to the reported procedures (Lin & Goddard, 2018b). In brief, a mixture of GMA-IDA, potassium persulfate, water, BPM, and n-butyl acrylate in the reaction vessel was purged with nitrogen gas with stirring for 20 min. After heating to 70°C, the reaction continued for 20 h in the dark with stirring, to produce the final copolymer, poly (2-propenoic acid,2-methyl-,3-[bis-(carboxymethyl) amino]-2-hydroxypropyl ester-*co*-*n*-butyl acrylate-*co*-4-benzoylphenyl methacrylate) (GMA-IDA-*co*-BA-*co*-BPM). Crude emulsion copolymer was then purified by centrifugation and dialysis in DI water, followed by dialysis in methanol. Dialysis was conducted using a 20 kDa regenerated cellulose membrane. The copolymer emulsion was stored in methanol at 4°C until use.

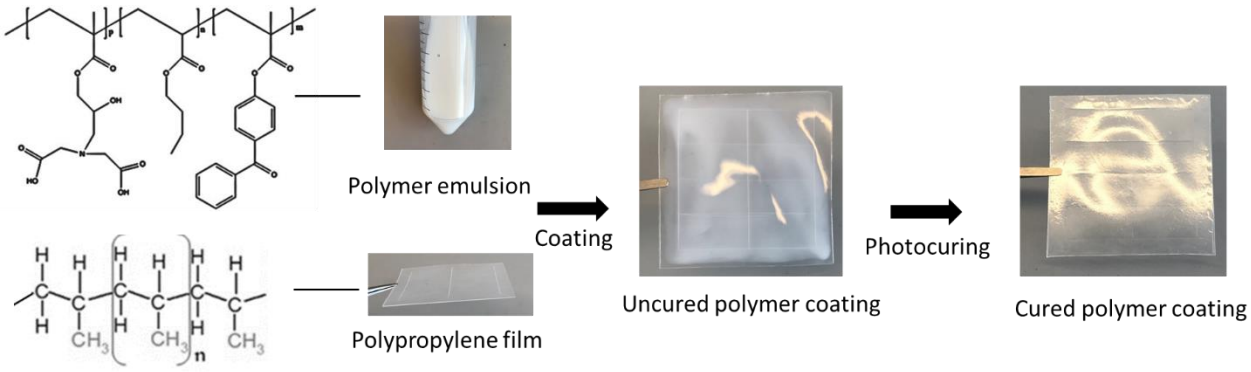

Figure 1. Preparation of non-migratory metal chelating active packaging. Emulsified copolymer, poly (2-propenoic acid,2-methyl-,3-[bis-(carboxymethyl) amino]-2-hydroxypropyl ester-co-n-butyl acrylate-co-4-benzoylphenyl methacrylate) (GMA-IDA-co-BA-co-BPM) is coated onto polypropylene films followed by photocuring.

Metal-chelating films were prepared in a coat-cure method, as illustrated in Figure 1. Emulsion copolymer (0.7 mL) was coated onto the surface of polypropylene coupons. After methanol evaporated, the coating was photocured under UV light (365 nm, approximately 205 mW/cm^2 fluence, Dymax 5000-EC Series, Torrington, CT) for 270 seconds. The metal-chelating films were washed in a water bath for 30 min at 60°C three times to remove residual monomers and loosely

adsorbed copolymer and dried in a desiccator. The resulting metal-chelating films were cut into 1 x 2 cm^2 for further analysis.

Characterization of material surface morphology & chemistry. Surface morphology was characterized using scanning electron microscopy (SEM). Samples were sputter-coated with gold (108 auto, Model No.7002-8, Ted Pella Inc, Redding, CA) and imaged at 15kV (JEOL, JCM 6000, Tokyo, Japan). Reported micrographs are representative of a total of eight images on quadruplicate films (two images collected at random locations on each film). The surface chemistry of native polypropylene and coated metal-chelating films was analyzed using attenuated total reflectance Fourier transform infrared (ATR−FTIR) spectroscopy on an IRTracer-100 FTIR spectrometer (Shimadzu Corporation, Kyoto, Japan) equipped with a diamond ATR crystal. Reported spectra are representative of spectra acquired on quadruplicate films at a resolution of 4 cm^{-1} (32 scans) with air as background spectrum.

Determination of metal chelating capacity in complex systems. The iron chelating capacity was determined by using inductively coupled plasma-mass spectroscopy (ICP-MS) using a modification of a previously reported method (Lin & Goddard, 2018b). To conduct ICP-MS analysis, native polypropylene and metal-chelating films were incubated in 0.08 mM ferric chloride solution in 50 mM sodium acetate/imidazole buffer at pH 4.0 in the dark for 96 hours. Films were rinsed with absolute ethanol, dried under pressurized house air, and dried in a desiccator. Two pieces of 1 x 2 cm^2 films (approximately 90 mg) were added to microwave digestion vessels (high purity Teflon vessels, Milestone S.r.l., Sorisole, Italy). The microwave digestion was conducted in 5 mL of nitric acid (trace metal grade) using the Ethos Microwave Digestion Labstation (Milestone S.r.l., Sorisole, Italy) (ramp to 210°C for 20 min, hold at 210°C for 20 min and cool down). Digested samples were transferred to 50 mL centrifuge tubes, diluted

with deionized water, and held at 4°C until analysis. ICP-MS analysis was conducted on a Thermo Scientific Element 2 Series ICP-MS (Thermo Fisher Scientific, Waltham, MA). Iron solutions (1000 ppm ICP−MS Standard, Sigma-Aldrich, St. Louis, MO) were used to prepare calibration standards. The effect of competing ions on ferric ion chelating capacity was investigated by addition of the following salts to the Fe^{3+} solution (0.08 mM ferric chloride in 0.05 M sodium acetate/imidazole, pH 4.0): 35 mM $CaCl_2$, 10 mM $MgCl_2$, or 0.8 M NaCl. Competing ion concentration was determined based on the concentration of target metal ions in milk, hard water, and salad dressing for calcium, magnesium, and sodium, respectively (Garzon & Eisenberg, 1998; Grimes, Nowson, & Lawrence, 2008; Hallberg, Rossander-Hultén, Brune, & Gleerup, 1992; Ogiwara et al., 2016). The effect of viscosity on ferric ion chelating capacity was investigated by addition of the following concentrations of methylcellulose (MC) to the Fe^{3+} solution (0.08 mM ferric chloride in 0.05 M sodium acetate/imidazole, pH 4.0): 0.25% MC, 0.5% MC, 1% MC, or 2% MC (Roman, Decker, & Goddard, 2015). The viscosity of each concentration of methylcellulose was measured with a Brookfield Dial Reading Viscometer with the electronic drive (Middleboro, MA), and concentrations were selected to simulate viscosities similar to cream, tomato juice, applesauce and salad cream, respectively.

Ascorbic acid degradation. Metal-chelating films were analyzed for their ability to control oxidative degradation of bioactive nutrients using a model ascorbic acid solution. One square centimeter metal chelating film was stored in each of the 10 mL sealed gas chromatography vials with 1 mL of 20 mM ascorbic acid in 10 mM sodium acetate/imidazole buffer at pH 4.0 at 37°C for 16 days without rotation. Blank ascorbic acid solutions, ascorbic acid solutions incubated with polypropylene, and ascorbic acid solutions with 0.08 mM EDTA (23.4 ppm equivalent, molecular weight 292.2 g/mol) were used as controls. According to FDA CFR Title 21, the limits for calcium

disodium EDTA are 75 ppm in salad dressing and sauces, and 33 ppm in carbonated soft drinks and alcoholic beverages (FDA, 2017). At each time point during the study, the ascorbic acid content of solutions incubated with metal-chelating films was determined using modified dichloroindophenol assay based on the Association of Official Analytical Chemists (AOAC) official method 967.21 with slight modification (Horwitz, 1970; Lin & Goddard, 2018b). Briefly, a 0.2 mL aliquot of the ascorbic acid solution was mixed with 4.8 mL of 0.04 wt % oxalic acid in water, and a 0.9 mL aliquot of the mixture was added to 4.1 mL of 0.2 mM 2,6-dichloroindophenol solution in water to yield a colored reaction product. The absorbance was measured immediately at 520 nm by Synergy Neo2 Hybrid Multi-Mode Reader (BioTek Instruments, Winooski, VT), and ascorbic acid content was calculated by comparing to a standard curve of ascorbic acid standards in 10 mM sodium acetate/imidazole buffer at pH 4.0. Data were fit to a first-order degradation rate equation to determine half-life and rate coefficients of ascorbic acid degradation. The effect of competing ions on antioxidant efficacy of metal-chelating films was investigated by addition of the following salts to the ascorbic acid solution (20 mM ascorbic acid in 10 mM sodium acetate/imidazole, pH 4.0): 35 mM $CaCl_2$, 10 mM $CaCl_2$, 10 mM $MgCl_2$, 10 mM NaCl or 0.8 M NaCl. The effect of viscosity on the performance of the metal chelating films in controlling ascorbic acid was determined by addition of methylcellulose to the ascorbic acid solution (20 mM ascorbic acid in 10 mM sodium acetate/imidazole, pH 4.0): 0.25% MC, 0.5% MC, 1% MC, or 2% MC. Films were submerged in 1 mL of each ascorbic acid solution in 10 mL GC vials and incubated at 37°C for 7 days without rotation. Remaining ascorbic acid contents were measured on day 0 and day 7 using the dichloroindophenol assay as described above.

Statistics. The preparation of metal-chelating films was conducted in duplicate batches. Surface analysis, metal chelating assays, and ascorbic acid degradation study were performed using

quadruplicate samples of metal chelating films prepared by a single representative batch of copolymer emulsion. Results of experiments are representative of at least two experiments repeated independently. Means from analysis of variance (ANOVA) analysis were compared using Tukey's multiple comparison tests ($P < 0.05$) in GraphPad Prism 6.0 (La Jolla, CA). Nonlinear fitting for ascorbic acid degradation was conducted in GraphPad Prism 6.0.

RESULTS & DISCUSSIONS

Surface Characterization

The copolymer latex was cast onto the surface of polypropylene and further photocured by exposure to UV-light to form a clear and glossy cured coating. The surface morphologies of the native and metal chelating films were observed under SEM (Figure 2A and 2B).

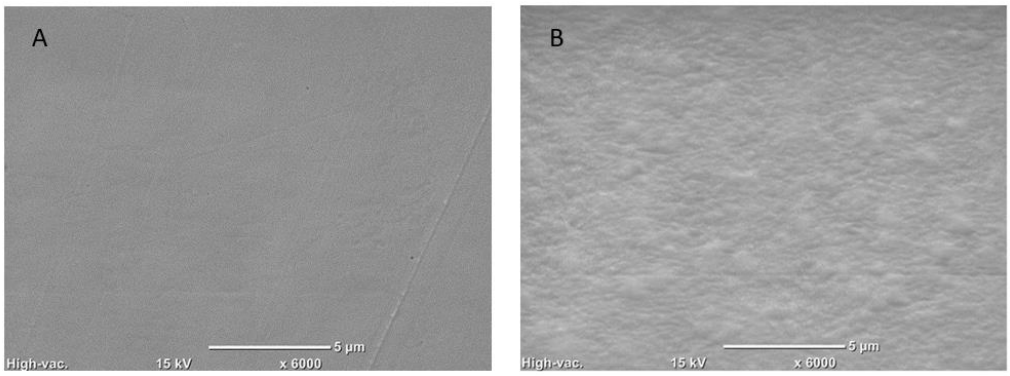

Figure 2. Electron micrographs of (A) native polypropylene, (B) metal-chelating film. Each image is representative of a total of four micrographs acquired at random locations on quadruplicate samples.

Metal chelating films exhibited a uniform surface. Introduction of the metal-chelating coating introduced a slight increase in surface roughness compared to the relatively smooth surface of native polypropylene. The observed change in surface morphology suggested that the metal-chelating copolymer had been successfully immobilized to the surface of the polypropylene films.

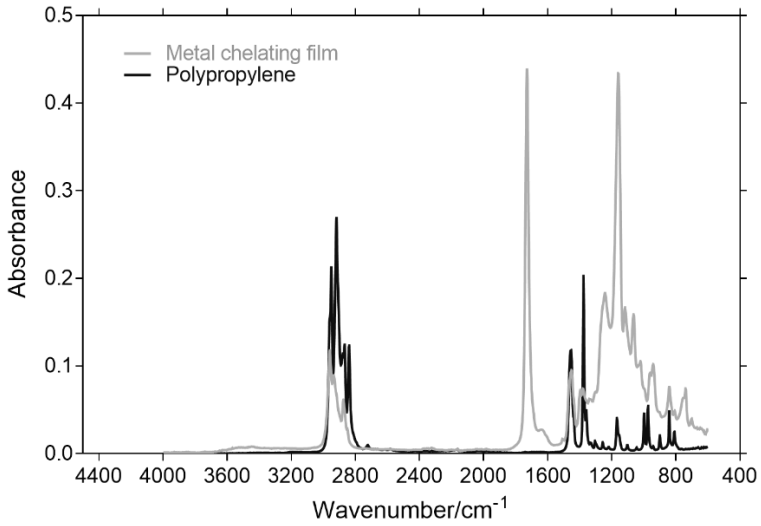

Figure 3. ATR-FTIR spectra of native polypropylene and metal-chelating film. Each spectrum is representative of a total of four spectra collected on quadruplicate samples.

The surface chemistry of the metal-chelating films was characterized by ATR-FTIR spectroscopy, with native polypropylene as the control (Figure 3). Native polypropylene exhibited characteristic absorption bands at 3000-2800 and 1500-1400 cm^{-1}, which were ascribed to alkane C−H stretching and bending vibrations, respectively. Metal-chelating films presented strong absorption bands at 1730 and 1240 cm^{-1}, indicating C=O and C−O−C stretching vibrations, respectively. The small band at 1637 cm^{-1} represented deprotonated IDA ligands, suggesting the presence of IDA ligands (Lin, Roman, Decker, & Goddard, 2016). These absorption bands are characteristic for the metal chelating material, in agreement with prior reports (Misra, Pichot, El-Aasser, & Vanderhoff, 1983; Suma, Jacob, & Joseph, 2010).

Influence of Competing Ion and Viscosity on Metal Chelation

The iron chelating capacity of the metal chelating material was quantified by analyzing the Fe^{3+} content using ICP-MS in the digest of the coated films (1 x 2 cm^2) which had been stored in 0.08 mM ferric buffered solutions at pH 4.0 for 96 hours at room temperature (25°C). Metal chelating films were prepared using 28 µL/cm^2 of copolymer emulsion and presented a ferric iron chelating

capacity of 95.6 ± 17.0 nmol/cm^2 at pH 4.0 while uncoated polypropylene chelated only 4.3 ± 0.7 nmol/cm^2 of ferric ion which is likely due to measurement error. These results suggest that native polypropylene does not adsorb iron and that the observed metal chelation in the metal chelating film is a result of ligand-specific interactions.

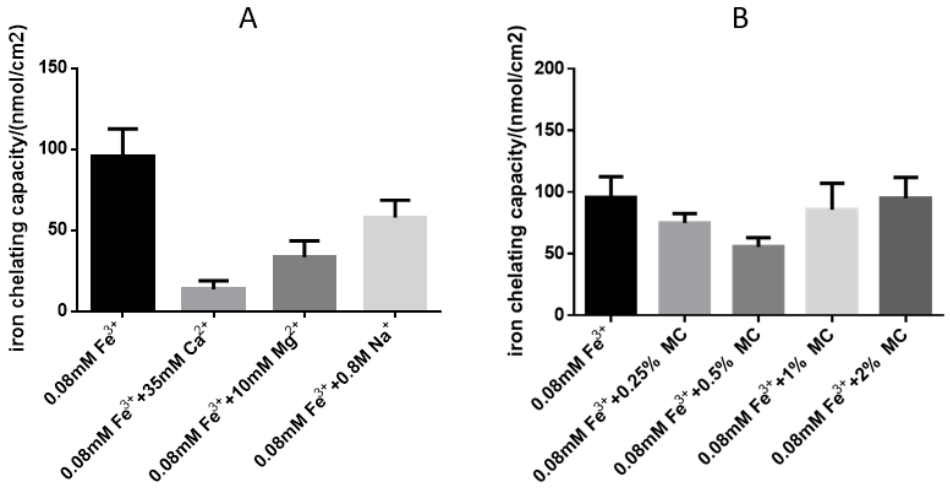

Figure 4. Effect of competing ions (A) and viscosity (B) on the iron chelating capacity of metal-chelating films. Viscosity was manipulated by the addition of methylcellulose. Error bars represent standard deviation (n=4).

The effect of competing ions and increasing viscosity on the metal chelating activity at pH 4.0 was further analyzed (Figure 4A and 4B). The amount of Fe^{3+} chelation decreased significantly in the presence of 35 mM Ca^{2+} or 10 mM Mg^{2+}, which might be explained by the stability constants of IDA for different ions. IDA is a tridentate metal chelator with three metal chelating sites including two carboxylates and one tertiary amine (Lin et al., 2016). The stability constants for IDA increase with increasing charge on the metal ion and decreasing radius of the metal ions (Smith & Martell, 1987). Therefore, IDA has high stability constants for Fe^{3+} (log K = 10.7) and Cu^{2+} (log K = 10.6), and low affinity for other ions common in foods, such as Ca^{2+} (log K = 2.6), Mg^{2+} (log K = 3.0), and Na^{+} (log K = 0.4) (Martell & Smith, 1974). Nevertheless, metal chelating film still retained 14% and 35% chelating capacity for calcium and magnesium, respectively. In term of sodium, no

significant difference in the amount of Fe^{3+} chelation was observed with the addition of 0.8 M Na^{+}. To increase viscosity, the ferric solutions were thickened with 0.25%, 0.5%, 1% and 2% methylcellulose. The apparent viscosity was then measured by viscometer at 100 rpm at 20 °C (Figure 5).

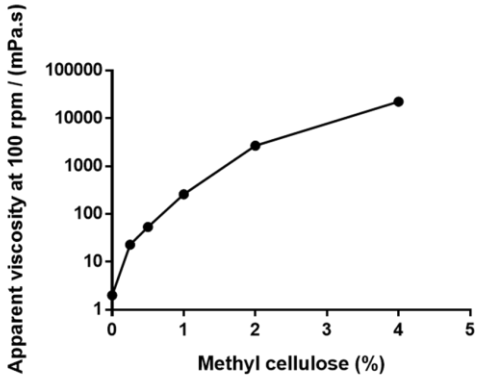

Figure 5. Apparent viscosity at 100 rpm of aqueous buffered solutions (50 mM sodium acetate/imidazole buffer, pH 4.0) thickened with methylcellulose.

According to statistical analysis, no significant difference between samples was observed despite the increase of viscosity up to 2% methylcellulose. It suggests that the active packaging material has the potential to be used viscous food systems as an alternative metal chelating system to prevent transition metal-induced oxidations because it retains efficacy at viscosities up to 2700 cP (centipoise), the viscosity of 2% methylcellulose and similar to the viscosity of salad cream.

Antioxidant Efficacy

The antioxidant efficacy of metal-chelating film was characterized by measuring its ability to hinder ascorbic acid degradation at pH 4.0. The degradation of ascorbic acid can take place in aerobic and/or anaerobic conditions and depends on factors such as temperature, pH, storage time and the presence of metallic ions (Fe^{3+} and Cu^{2+}) (Bosch et al., 2013). By the aerobic pathway, L-ascorbic acid is oxidized to dehydroascorbic acid, which is less stable and can degrade in different ways (Peleg, Normand, Dixon, & Goulette, 2018). According to previously reported studies, the

metal chelating materials controlled ascorbic acid degradation in aqueous solution by scavenging transition metals (Lin & Goddard, 2018a, 2018b; Lin et al., 2016).

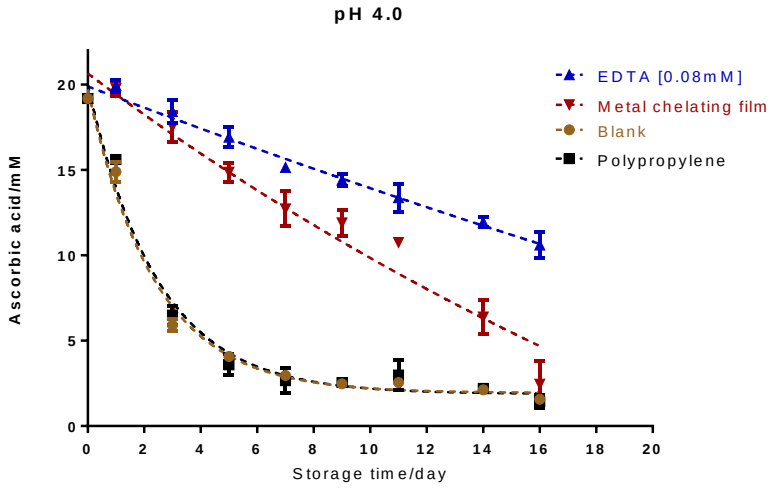

Figure 6. Control of ascorbic acid degradation by metal-chelating films at pH 4.0 (stored at 37°C) over 16 days. Values represent means with error bars indicating standard deviation (n=4). In some instances, error bars are smaller than data points.

A storage study was performed in which metal chelating films were stored in solutions of ascorbic acid at pH 4.0 and 37°C, and ascorbic acid content was measured at each time point over 16 days (Figure 6). The blank ascorbic acid solution, the ascorbic acid solution with native polypropylene films, and the ascorbic acid solution containing EDTA were prepared as controls. The results showed that the ascorbic acid degradation of blank and polypropylene can be successfully described by a 2^{nd}-order kinetics when oxygen is present in a limited amount (Baiano, Marchitelli, Tamagnone, & Nobile, 2004). However, for ascorbic acid degradation of EDTA and metal chelating film, a first-order model was followed as degradation was much slower and oxygen was not yet a constraint, in agreement with previous reports (Assiry, Sastry, & Samaranayake, 2006; Li, Yang, Yu, & Wang, 2016). At pH 4.0, ascorbic acid solution alone (negative control) and with native polypropylene showed similar ascorbic acid degradation kinetics with a calculated shortest degradation half-life of 2 days. On the other hand, when incubated with metal chelating films,

degradation was significantly slowed, extending the degradation half-life to 10 days. EDTA was the most effective against ascorbic acid degradation at pH 4.0 and retained about 50% of the ascorbic acid on day 16. These results suggest that while EDTA remains the most effective at controlling ascorbic acid degradation, the metal-chelating coating extended the degradation half-life from 2 to 10 days, indicating its potential to be used as an antioxidant active packaging material to control the degradation of transition metal-induced oxidation.

Influence of Competing Ions on Antioxidant Efficacy

The influence of competing ions and viscosity on the antioxidant efficacy of metal chelating films was determined by characterizing their influence on controlling degradation of ascorbic acid at 37°C and pH 4.0 for 7 days. Ascorbic acid solutions containing 35 mM Ca^{2+}, 10 mM Mg^{2+}, and 0.8 M Na^{+} were prepared and the ascorbic acid contents on day 0 and day 7 were measured (Figure 7A). Compared to the result of blank without competing ions, it is observed that 313%, 159%, and 380% more ascorbic acid was left on day 7 when the storage study was performed in solutions with 35 mM Ca^{2+}, 10 mM Mg^{2+} and 0.8 N Na^{+}, respectively. Previous reports showed that sodium chloride (0.1 M) at pH 7.0 has an inhibiting effect on the oxidation of ascorbic acid by a hypothesized mechanism of inhibiting the catalytic power of copper (Høygaard & Rasmussen, 1938; Kellie & Zilva, 1935). Another study also found that by using an accelerated stability test, the addition of Mg(II) ions (1 mg/L) to 0.3 mM ascorbic acid significantly increases its stability (Dolińska et al., 2012). Although not found in aqueous solutions, calcium presence in edible films stabilized ascorbic acid and slowed down the browning development because of higher water immobilization (León, Lamanna, Gerschenson, & Rojas, 2008; Pérez, Fissore, Gerschenson, Cameron, & Rojas, 2012).

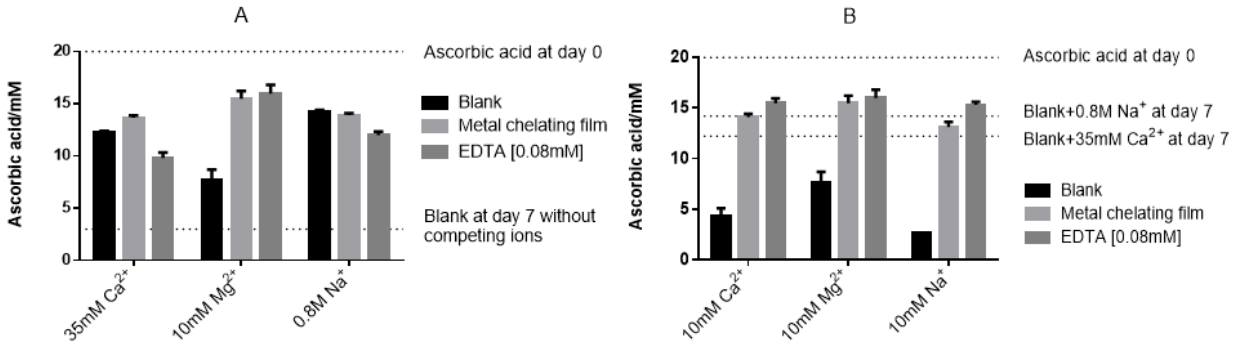

Figure 7. Effect of high concentration (A) and low concentration (B) of competing ions (Ca, Mg, Na) on the metal chelating films' ability to control ascorbic acid degradation after storing at 37°C for 7 days. Error bars represent standard deviation (n=4).

This inhibiting phenomenon was further studied by reducing calcium and sodium to the same concentration of magnesium which was 10 mM (Figure 7B). After reducing the concentration of calcium chloride and sodium chloride, the ascorbic acid content in blank on day 7 sharply dropped to the similar level of that without competing ions which was around 4 mM (20%), confirming that high concentration of salts can inhibit the degradation of ascorbic acid to certain degrees. The introduction of competing ions did not affect the antioxidant efficacy of metal chelating films even after changing the concentration of calcium and sodium, with about 70% ascorbic acid remained on day 7. It suggests that metal chelating film retained its antioxidant efficacy in the presence of calcium, sodium, and magnesium. On the other hand, the addition of 35 mM calcium and 0.8 M sodium significantly dropped the ascorbic acid retention with EDTA while adding magnesium did not have a significant effect. However, when a reduced amount of calcium and sodium were added to the buffer, the ascorbic acid content with EDTA was not significantly changed. Calcium can be chelated to the EDTA by six bonds and $CaEDTA^{-2}$ is very stable, therefore, less ETDA was available to chelate transition metals which resulted in accelerated ascorbic acid degradation. Magnesium can also be connected to the EDTA, but in this case, the concentration of magnesium was too low to make a difference. It also explained why EDTA's performance was not significantly

affected when a lower concentration of calcium and sodium were present. Nevertheless, metal chelating film still outperformed EDTA when 35 mM Ca^{2+} or 0.8 N Na^{+} was added. In summary, metal chelating films retained 100% efficacy in the presence of competing ions (Ca^{2+}, Mg^{2+}, and Na^{+}) up to the concentration used in this experiment and has the potential to replace EDTA in food with a high concentration of salts.

Effect of Viscosity on Antioxidant Efficacy

The antioxidant performance of metal chelating film at a series of viscosities thickened by methylcellulose was characterized by studying the ascorbic acid remained on day 7 (Figure 8).

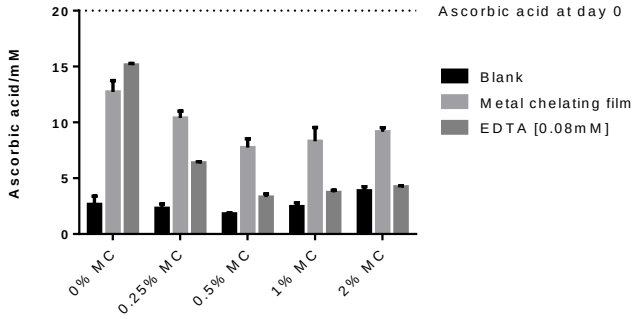

Figure 8. Effect of viscosity on the metal chelating films' ability to control ascorbic acid degradation after storing at 37°C for 7 days. Error bars represent standard deviation (n=4).

Around 18% ascorbic acid was left in blank on day 7 with no statistical difference found as the viscosity increased. Metal chelating film retained at least 61% antioxidant efficacy at viscosities up to 2700 cP (2% methylcellulose), with the lowest ascorbic acid content of 7.7 mM at 0.5 % methylcellulose. In contrast, the addition of methylcellulose significantly decreased the ascorbic acid remained in the solution with EDTA on day 7, lowering the remained ascorbic acid from approximately 75% to 20%. To sum up, metal chelating film remarkably outperformed EDTA as the viscosity of solution increased, which suggests that the photocured metal chelating copolymer coating could potentially substitute EDTA in viscous foods to control transition metal-induced oxidations.

CONCLUSIONS

In this work, we investigated the iron-chelating capacity and antioxidant efficacy of the GMA-IDA-*co*-BA-*co*-BPM copolymer which was synthesized via emulsion polymerization and applied on polypropylene films via coating/photocuring preparation. The SEM image showed the successful application of the copolymer on the film and the FTIR spectrum revealed the presence of IDA ligands and C=O and C−O−C stretching vibrations, which are characteristic for metal chelating film. In the presence of 35 mM calcium and 10 mM magnesium, the iron-chelating capacity of metal-chelating film significantly dropped while was not significantly affected by the addition of 0.8 M sodium. Metal chelating film also retained its chelating capacity at viscosities up to 2700 cP (2% methylcellulose), similar to the viscosity of salad cream. In addition, metal chelating film exhibited a high retention of antioxidant efficacy as an effect against transition metal-induced ascorbic acid degradation even when competing ions were present or in solutions of moderate viscosity (2700 cP), significantly outperforming EDTA. In summary, the GMA-IDA-*co*-BA-*co*-BPM copolymer can be applied on common packaging materials with a simple process and retained iron-chelating capacity and antioxidant efficacy even in the presence of competing ions (calcium, magnesium, and sodium) and increasing viscosities. Thus, this metal-chelating active coating has the potential to substitute EDTA in foods with a high concentration of salts or moderate viscosity.

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